Laparoscopic abomasal cannulation in sheep

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ABSTRACT: Abomasal cannulation, an important research tool in experimental procedures, provides a method for the placement of an abomasal cannula in sheep. The aim of this study was to describe a technique for laparoscopic abomasal cannulation in sheep. It was performed in six anaesthetised sheep positioned in dorsal recumbency using three portals in the abdomen. The first absorbable traction suture was placed 1 cm cranial to the determined abomasal cannula site. A stab incision was made with a laparoscopic monopolar hook electrode in the middle of a purse-string suture placed around the abomasal cannula site. The T-shaped cannula was inserted into the abomasum lumen through the stab incision, and the second traction suture was then placed 1 cm caudal to the abomasal cannula site. The T-shaped abomasal cannula was pulled out of the abdominal cavity through the exit wound located 3–5 cm lateral and 10–12 cm cranial to the right side of the *umbilicus*. The two traction sutures were tied. The T-shaped cannula was secured to the skin with a finger-trap suture. Repeat laparoscopy was performed 1 month later. Firm adhesion between the abomasum and abdominal wall was observed in all sheep, with no evidence of leakage or peritonitis. No major intraoperative or postoperative complications were encountered. The median surgical time was 49 min, with a range from 42 to 58 min. The abomasal contents were collected easily. In conclusion, laparoscopic abomasal cannulation is safe and easy to perform. Its low complication rates and the "ideal" placement of the tube into the abomasum make it an especially attractive alternative to traditional surgical abomasal cannulation in veterinary practice.

Keywords: laparoscopy; ewes; abomasum; T-shaped abomasal cannula

The cannulation of various regions of the digestive tract of ruminants is an important research tool (Johnson 1966; Knight et al. 2010; Kristensen et al. 2010; Allen et al. 2011). There has been considerable interest in the cannulation of the abomasum in nutritional studies to obtain abomasal fluid for the measurement of the abomasal contents (Pearson et al. 1981) and to determine the outflow rate of abomasal fluid for the further study of abomasal function (Holtenius et al. 2000). The application of abomasal cannulation has also been described for the examination of the abomasum in follow-up studies in veterinary parasitology (Hertzberg et al. 1994) and for the infusion of nutrients and drugs into the abomasum to reach high concentrations (Charles et al. 1993; Vynckier and Debackere 1993). All these studies involved previous fitting with a cannula in the abomasum.

In sheep, access to the abomasum has been achieved through celiotomy with the exteriorisation of the abomasum, but this technique is invasive, requiring a right flank laparotomy, and the abdomen is typically closed in three layers (Driedger et al. 1970). Laparoscopic procedures are minimally invasive surgical techniques that allow excellent observation, minimal incision, lower complication rates, rapid postoperative recovery, lower pain scores and improved patient convalescence compared with open surgical procedures (Davidson et al. 2004; Babkine and Desrochers 2005; Hendrickson 2008; Jimenez Pelaez et al. 2008; Dupre et al. 2009). We are not aware of any previously reported method for laparoscopic abomasal cannulation in sheep. Thus, the object of this study was to develop a simple and effective surgical technique for abomasal cannulation using a laparoscopic technique in sheep.

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MATERIAL AND METHODS

Animals. Six non-pregnant northeast semi fine wool eves, one to two years of age and weighing 38 to 43 kg, were used in this study. All sheep were assumed to be healthy based on physical examination and complete blood count (CBC). Sheep were kept under an intensive system of management, receiving corn silage and balanced feed diet twice a day, and had access to water and mineral salt *ad libitum*. The study was approved by the Animal Ethics Committee of the Northeast Agricultural University (Harbin, China) to ensure adherence to the Canadian Council on Animal Care guidelines.

T-shaped abomasal cannula. A silicone-coated latex catheter with an internal diameter of 7.3 mm and an external diameter of 9.3 mm was purchased ready-made in the form of a T (Star Enterprise Co Ltd, Zhanjiang, China). The barrel of the cannula to exteriorise through the abdominal wall was cut to 10 cm in length. The top of the "T" for insertion in the lumen of the abomasum was cut in an ellipse shape with a length of 4.5 cm and sides that extended one-half of the diameter of the catheter at the "T" and was narrowed down to rounded ends at both extremes of the ellipse shape. The peripheral edge of the top of the "T" portion was smoothed using scissors to facilitate the flow of digesta. The barrel of the cannula was temporarily blocked with a sterilised tampon before inserting.

Surgical procedure. Prior to the procedure, food was withheld from sheep for 36 h and water for 12 h. Ampicillin sodium (Harbin Pharmaceutical Group, Harbin, China, 40 mg/kg, i.m.) was administered 1 h prior to surgery. Each sheep was pre-medicated with atropine (atropine sulphate injection, Hainan Pharma Co Ltd, Hainan, China) at 0.05 mg/kg subcutaneously, and approximately 15 min later was administered with ketamine at 22 mg/kg and xylazine at 0.2 mg/kg, intramuscularly (Ozkan et al. 2010). Sheep were positioned in dorsal recumbency and the surgical table was tilted to between 10°-20° in a head-down position. The ventral abdomen, from the paracostal area to the pubis and to each inguinal fold, was shaved, aseptically prepared, and draped for surgery.

The ventral abdomen was approached through three portals (Figure 1): the laparoscope portal (portal 1) and two instrument portals (portal 2 and portal 3). For the laparoscope portal, a stab incision was made through the body wall on the linea alba

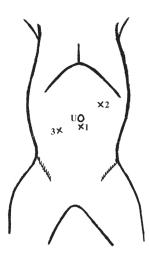


Figure 1. Portal locations and the exit wound of the T-shaped abomasal cannula. Portal one (laparoscope) was on the linea alba 2 cm caudal to the *umbilicus* (U). Portal 2 (needle driver) was 12–16 cm lateral and 4–5 cm cranial to the left side of the *umbilicus*. Portal 3 (left-curved preparation forceps) was 12–16 cm lateral and 2–3 cm caudal to the right side of the *umbilicus*. EW = the exit wound of the T-shaped abomasal cannula

2 cm caudal to the *umbilicus*. The surgeon and one assistant elevated the ventral abdominal wall and provided counter pressure while a 10/11 mm trocarcannula unit (blunt, Optical Medical Instrument Co Ltd, Hangzhou, China) was inserted into the abdomen (portal 1), and the cannula was connected to an automatic high-flow CO2 insufflator (Olympus, Hamburg, Germany) to insufflate the abdominal cavity to an intra-abdominal pressure of 8 mm Hg with carbon dioxide and to maintain this value. Then, the blunt trocar was removed and replaced by a 0° laparoscope (10 mm in diameter, 330 mm long, Olympus, Hamburg, Germany) with a videoendoscopic camera and light source (Olympus, Hamburg, Germany). A brief exploration of the abdominal cavity was performed. The abomasum and associated structures could be observed under the guidance of the laparoscope. After laparoscopic inspection, a 10-mm instrument portal was created 12-16 cm lateral and 4-5 cm cranial to the left side of the *umbilicus* (portal 2). A 5-mm instrument portal was created 12-16 cm lateral and 2-3 cm caudal to the right side of the *umbilicus* (portal 3). A 10-mm laparoscopic needle driver (330 mm long, Olympus, Hamburg, Germany) and a 5-mm left-curved preparation forceps (330 mm long, Olympus, Hamburg, Germany) were introduced through portal 2 and portal 3, respectively.

The accessory trocar-cannula units were inserted through abdominal stab incisions under laparoscopic guidance.

The serosal surface of the greater curvature of the abomasum was identified. Using forceps, the abomasum was grasped in the middle of its greater curvature (midway between the reticulo-abomasal ligament and the pyloric antrum), 2 to 3 cm from the attachment of the greater omentum. This site was the location of the cannula in the abomasum.

Absorbable suture material (USP 2-0 polyglycolic acid, 30 cm, Shanghai Pudong Jinhuan Medical Products Co Ltd, Shanghai, China) swaged onto a needle was used for the two traction sutures and one purse-string suture. The needle was made to three-eighths circle curved. The location of the first traction suture was between the *umbilicus* and the xyphoid process, 3-5 cm lateral and 12-14 cm cranial to the right side of the umbilicus. The needle was passed through the abdominal wall via a 1 cm cutaneous incision and grasped within the peritoneal cavity by the laparoscopic needle driver, and then passed through the abomasal wall approximately 1 cm cranial to the abomasal cannula site without mucosal penetration to achieve a bite of ~ 2 cm, perpendicular to the great curvature of the abomasum. Then, the needle was pulled out of the abdominal cavity to create the first traction suture. The two ends of the traction suture material were secured outside the abdomen using haemostatic forceps without applying any tension. The traction suture was performed on the greater curvature of the abomasum after the abomasum was placed in its anatomical correct position.

Absorbable suture material (USP 2-0 polyglycolic acid) swaged onto a three-eighths circle curved needle was inserted intra-abdominally with a laparoscopic needle driver through portal 2. A partial thickness purse-string suture (1 cm in diameter) was placed around the abomasal cannula site.

The laparoscopic needle driver was removed from portal 2 and a 5 mm converter was connected with the 10 mm laparoscopic cannula; then, the laparoscopic monopolar hook electrode (5 mm in diameter, 330 mm long; Olympus, Hamburg, Germany) was inserted into portal 2. Subsequently, a perforating stab incision a little smaller than the diameter of the T-shaped abomasal cannula was made into the abomasal wall in the centre of the purse-string suture with laparoscopic monopolar hook electrode (Figure 2). The creation of the

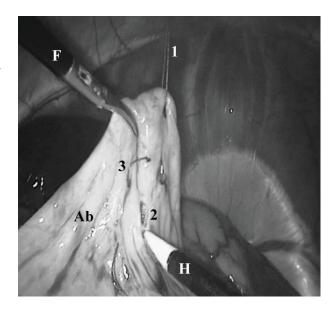


Figure 2. Intraoperative view. The abomasal wall in the centre of the purse-string suture was perforated completely using a laparoscopic monopolar hook electrode (H). 1 = the first absorbable traction suture; 2 = stab incision; 3 = purse-string suture; F = left-curved preparation forceps; F = Ab = abomasum

stab incision was performed under the traction of the first traction suture and the laparoscopic leftcurved preparation forceps.

The T-shaped abomasal cannula pre-blocked with a sterilised tampon was completely inserted into the abdominal cavity through the lumen of the 10 mm laparoscopic cannula (portal 1). The laparoscopic monopolar hook electrode was replaced by a second laparoscopic left-curved preparation forceps. Then, the abomasal cannula was inserted into the abomasum lumen through the stab incision with the assistance of the second laparoscopic left-curved preparation forceps and the purse-string suture was tightened. The placement of the abomasal cannula was performed under the traction of the first traction suture and the first laparoscopic left-curved preparation forceps.

The second traction suture was placed 1 cm caudal to the abomasal cannula site using the same technique and the same suture material as the first traction suture along the greater curvature of the abomasums (Figure 3). The second traction suture was located between the umbilicus and the xiphoid process, 3–5 cm lateral and 7–9 cm cranial to the right side of the *umbilicus*.

In the next step, the exit wound of the T-shaped abomasal cannula in the corresponding abdominal

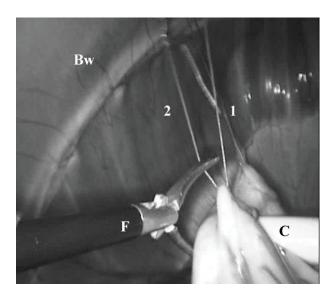


Figure 3. Intraoperative view. The second traction suture was placed 1 cm caudal to the abomasal cannula site. 1 = the first absorbable traction suture; 2 = the second absorbable traction suture; Bw = body wall; F = left-curved preparation forceps; C = T-shaped abomasal cannula

wall was created. It was located 3–5 cm lateral and 10–12 cm cranial to the right side of the *umbilicus*. A stab incision was made through the body wall at the exit site, and the haemostatic forceps were used to penetrate the wall. The external portion of the T-shaped abomasal cannula was pulled through the exit and out of the abdominal cavity using the haemostatic forceps. The correct positioning of the abomasum was verified.

Carbon dioxide was evacuated from the abdominal cavity by opening the cannulas, and the T-shaped abomasal cannula was pulled against the body wall while the abomasum was approximated to the body wall by pulling on the traction sutures. The two traction sutures were tied and buried under the skin, which was closed with a cruciate suture pattern (2-0 polyglycolic acid) to perform an abomasopexy. The T-shaped abomasal cannula was secured to the skin with a finger-trap suture and was clamped after removal of the sterilised tampon.

The laparoscopic cannulas were removed after the abdomen was decompressed. The 10 mm portals were closed in two layers and the 5 mm portal in one layer using silk. The operative time and any intraoperative and postoperative complications were recorded.

Postoperative care and postoperative monitoring. Postoperative care consisted of administering ampicillin sodium (40 mg/kg, *i.m.*) three times a day

for five days and flunixin meglumine (0.5 mg/kg, *i.m.* Harbin Pharmaceutical Group, Harbin, China) once daily for three days. Food was withheld for 12 h after surgery, followed by feeding fodder mixed with water to make a paste. Later, there was a gradual transition to dry feed. Postoperative monitoring included subjective assessment of the sheep (general attitude, appetite and the condition of the abomasal cannulation site), and objective evaluation of its general heath based on the physical examination (rectal temperature, heart rate and respiratory rate) once a day for seven days.

Collection of abomasal contents and disposal of abomasal cannulae. Abomasal contents were collected following clamp removal from the cannula barrel three times a day for 20 days, in a four-day cycle. Abomasal contents were collected at 08:00, 16:00, and 24:00 on the first day, at 06:00, 14:00, and 22:00 on the second day, at 04:00, 12:00, and 20:00 on the third day, and at 02:00, 10:00, and 18:00 on the fourth day. The T-shaped abomasal cannulae were removed from all sheep 20 days after surgery. Surgical removal was not required. After removal of the finger-trap suture, the abomasal cannulae could be safely pulled out by simple traction of the cannula barrel. Observation of the cannulation sites with respect to wound healing or leakage of abomasal contents was continued for 10 days.

Second look laparoscopy for evaluation of the abomasal cannulation site. Laparoscopic examination to re-evaluate the entire abdominal cavity and the abomasal cannulation site was performed one month after surgery in the same manner as the initial laparoscopy, but without the preparation of instrument portals. The presence or absence of adhesions, as well as their appearance at the cannulation site, were confirmed by laparoscopic visualisation.

Statistical analysis. The data were calculated as the mean \pm SD and analysed using the statistical software SPSS v13.0 for Windows (SPSS Inc., Chicago, IL, USA). Data were analysed using one-way ANOVA followed by the Student-Newman-Keuls test. Differences were considered significant at P < 0.05.

RESULTS

We successfully performed abomasal cannulation in six healthy sheep using a laparoscopic technique. Laparoscopic abomasal cannulation in sheep required only two 10 mm and one 5 mm incisions

in the body wall; the total length of all incisions was 2.5 cm. Mean surgical time (defined as time from the initial stab incision to closure of the last portal) was 49 min and ranged from 42 to 58 min. No complications occurred during insertion of the trocar-cannula units. Portals were placed too close together in one sheep, leading to problems in the manipulation of the laparoscope and instruments. The haemorrhage was negligible during the incision of the abomasal wall with a monopolar hook electrode. There was no spillage of abomasal contents under the traction of the first traction suture and the laparoscopic left-curved preparation forceps, demonstrating that peritoneal contamination can be reliably avoided. The results of physical examination are shown in Table 1.

The T-shaped abomasal cannula was useful for the sampling of abomasal contents throughout the duration of the study. Each sample of abomasal content was 20 g. There were sometimes obstructions of the cannula during abomasal content collection, in which case the T-shaped abomasal cannula was flushed with warm water. The T-shaped abomasal cannulae were held tightly in the abomasum lumen, and the purse-string resulted in a tight seal. No accidental T-shaped cannula dislodgment or damage was observed. There was no indication of intraperitoneal abomasal content leakage during T-shaped abomasal cannula placement in any sheep.

Removal of the abomasal cannula was performed easily by simple traction, with no associated adverse events. The fistulae healed seven days after the removal of the abomasal cannulae, and no leakage of abomasal contents was observed past 10 days of observation.

Repeat laparoscopy was performed one month postoperatively in the same manner as the initial

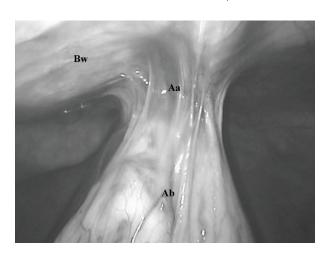


Figure 4. Adhesion one month after laparoscopic abomasal cannulation. Bw = body wall; Ab = abomasum; Aa = adhesion area

laparoscopy but with the laparoscope portal only. Firm adhesion was evident between the outer serosal surface of the abomasum and the parietal peritoneum at the site where the barrel of the cannula was exteriorised through the abdominal wall. There was no evidence of internal digesta leakage or peritonitis in any sheep (Figure 4). No apparent adverse effects of the T-shaped abomasal cannula were observed. Adhesion between the omentum and the abomasopexy site was observed in only one sheep, and no other abdominal abnormalities could be identified.

DISCUSSION

The sheep has become the model of choice for numerous areas of research, especially in animal nutrition (Knight et al. 2010). Studies of nutrient

Table 1. Mean (± SD) results of physiological examination after laparoscopic abomasal cannulation in sheep

	Heart rate (min)	Respiratory rate (min)	Body temperature (°C)
Before surgery (day)	75.50 ± 1.64	23.83 ± 2.64	38.93 ± 0.31
1	76.50 ± 2.88	24.17 ± 1.72	39.17 ± 0.34
2	74.50 ± 1.87	23.67 ± 2.16	39.03 ± 0.24
3	74.67 ± 1.86	22.83 ± 3.06	39.05 ± 0.36
4	75.17 ± 2.32	21.00 ± 2.61*	38.82 ± 0.47
5	74.67 ± 1.51	23.83 ± 2.14	39.05 ± 0.31
6	73.83 ± 1.94	22.00 ± 2.19	38.95 ± 0.32
7	75.00 ± 1.79	24.00 ± 1.79	39.02 ± 0.34

Significant differences from before surgery, *P < 0.05

availability and metabolism often require access to the gastrointestinal tract of sheep. However, these methods frequently require major surgical manipulation for placement. Similar to human medicine, laparoscopic techniques have undergone rapid development in veterinary medicine because of their minimal invasiveness. Laparoscopy has experienced a transition from a diagnostic tool to a prophylactic procedure and more recently to a therapeutic procedure (Freeman 2009). To our knowledge, no previously reported studies in the literature applied laparoscopic techniques to establish an experimental model for empirical studies, and we developed a prospective study to assess the feasibility. In this study, we performed laparoscopic abomasal cannulation in six healthy sheep with no fatal complications. The most common complications, such as splenic and intestinal puncture, occur during insertion of the trocar-cannula (Desmaizières et al. 2003). In this study, there was no injury to the spleen or bowel puncture, and bleeding was negligible during the placement of the trocar-cannula. Complications following a laparoscopic procedure are rare (Seeger et al. 2006), and in our study, the results of physical examination showed no statistically significant differences before and after surgery. However, regular monitoring of the physiological parameters is important to detect potential complications at an early stage. The amounts of abomasal content were 20 g, which was sufficient for a nutrition study.

The human experience of laparoscopy suggests that it is a painful procedure (Borkar and Dave 2005), which is also likely to be the case with sheep. It is assumed that the use of a general anaesthetic was a distressing and probably painful experience, and most of the pain arising during the laparoscopy procedure is due to stretching of the peritoneum, caused by distension of the abdomen with CO_2 (Stafford et al. 2006). The postoperative use of nonsteroidal anti-inflammatory drugs could have helped to reduce the distress response caused by laparoscopy and facilitate a faster return to the normal condition.

In the experience of some authors, the abomasal emptying rate is decreased in cows with left displaced abomasums and is further decreased immediately after the surgical correction of left displaced abomasums (Wittek et al. 2008; Constable et al. 2012). Whether this is also the case in sheep after abomasal cannulation remains uncertain. In our study, we did not investigate the postoperative abomasal

emptying rate, but all the sheep were comfortable after surgery, as perceived based on their satisfactory appetite. Similarly, a significantly higher feed intake after surgery in cows treated using the laparoscopic abomasopexy procedure has been described by other authors (Seeger et al. 2006). For our small number of animals, considering that antimicrobials can also reduce appetite, the prophylactic use of prokinetic drugs is recommended.

The location of the portals was an important contributing factor to the ease of surgery (Cokelaere et al. 2005). Laparoscopic abomasal cannulation was performed through a triangular disposition of three portals: the two instrument portals and the laparoscope portal. The location of the laparoscope portal allowed visualisation of the abomasum and associated structures, facilitating the manipulation of the abomasum. In this study, the somatotypes of the sheep were large, and the abdominal cavity was large after insufflation. It is therefore suggested that the instruments must have adequate length to reach the abomasal wall easily. In addition, the location of the instrument portals should not be too close to the abomasum, which would interfere with the surgical procedure.

Many surgeons have reported on the materials used for the abomasal cannula. Some surgeons (Dougherty 1955) have described the application of rigid plastic cannula, while some (Driedger et al. 1970) found a cannula made of rigid materials unsatisfactory and described the use of silicone medical-grade tubing for rumen and abomasal cannulae. They also reported that larger diameter abomasal cannulae were found to clog less frequently than small diameter catheters. In this study, the 22 F Latex T-shaped catheter was modified to make a T-shaped abomasal cannula that stuck tightly in the abomasum lumen. The internal diameter of the T-shaped cannula was 7.3 mm. It was easy to withdraw abomasal contents, and the insertion of the T-shaped cannula was convenient.

The middle of the greater curvature of the abomasum (midway between the reticulo-abomasal ligament and the pyloric antrum), 2 to 3 cm from the attachment of the greater omentum, was selected to be the T-shaped abomasal cannula site, which facilitated the collection of the abomasal contents. This site was out of the influence of gravity such that the T-shaped cannula could remain *in situ*. A laparoscopic monopolar hook electrode was used to penetrate the abomasum lumen with minimal

damage to the abomasum and negligible bleeding. The incision into the abomasum lumen was smaller than the diameter of the T-shaped cannula, which resulted in a tight seal for the T-shaped cannula using a purse-string suture.

To perform laparoscopic abomasal cannulation, two traction sutures were placed between the abdominal wall and the abomasal wall. The traction sutures were made through the body wall as described by Babkine et al. (2006). The first traction suture was made before the insertion of the T-shaped cannula to facilitate the surgical procedure and to reduce the risk of leakage of abomasal contents into the abdomen. The second traction suture was made after the insertion of the T-shaped cannula to create a secure fixation between the abomasum and the ventral body wall with the first traction suture.

Precautions were taken to prevent spillage of the abomasal fluid into the abdominal cavity during the incision of the abomasal wall and the placement of the abomasal cannula. First, food was withheld for 48 h and water for 12 h. Second, the administration of atropine before surgery caused abomasal atony and thus helped to prevent leakage by interfering with motility. Third, the incision of the abomasal wall and the placement of the abomasal cannula were under the traction of the first traction suture and the laparoscopic left-curved preparation forceps. Fourth, a laparoscopic monopolar hook electrode was used to penetrate the abomasum lumen with minimal damage and negligible bleeding. Fifth, the T-shaped abomasal cannula was obstructed with a sterilised tampon during placement.

Laparoscopy is simple to perform and considered to be safe, with few complications, but laparoscopic equipment is expensive (Monnet and Twedt 2003). In small animal clinics, laparoscopy is more expensive than general surgery. However, due to the minimal invasiveness of the procedure and the rapid postoperative recovery of the animal, this procedure has been used increasingly in small animal clinics (Kim et al. 2011). The economic problem is also encountered in sheep. However, abomasal cannulation of sheep has become the method of choice for numerous areas of research. The laparoscopic placement of catheters offers an alternative to open surgical gastrointestinal tract cannula placement, and the minimal invasiveness of this procedure can improve the accuracy of research. Given these factors and also as a result of increasing economic development, this procedure is likely to be used in sheep more and more.

An inflammatory response after abomasal cannulation was diagnosed on the basis of clinical signs (fever, tenseness of the abdominal wall, and moderate decrease in general condition), and transabdominal ultrasonography would be required to confirm peritonitis. The T-shaped abomasal cannulae were removed from all sheep 20 days after surgery with simple traction as described by Ahmed et al. (2005), and second look laparoscopy revealed no evidence of peritonitis or leakage of digesta. The presence of a large number of fibroblasts has been reported to be responsible for the formation of adhesions (Schreinemacher et al. 2009). Both the peritoneal injury and the presence of foreign material enhance adhesion formation: these factors induce increased vascular permeability followed by an inflammatory response with a simultaneous release of inflammatory proteins (Zinther et al. 2010). Several investigations have revealed that surgery leads to ingrowths of fibroblasts into the fibrin matrix, depositing collagen and resulting in the formation of connective tissue and adhesion (fibrous bands) between the injured peritoneal surfaces (Holmdahl 1997; Duron 2007). As the aim of our study was to investigate the feasibility of laparoscopic abomasal cannulation, which would be an important research tool in animal nutrition research, and most such research ends before removal of the cannula, we did not investigate the time of persistence of the adhesions afterwards.

In conclusion, laparoscopic abomasal cannulation facilitated short-term placement of abomasal cannula. Invasiveness is often measured by length of incision, severity of pain perceived, or recovery time. These factors are prone to biased reporting by enthusiastic surgeons and are difficult to measure in the animal environment (Bingener et al. 2009). The determination of systemic inflammation has been utilised to provide a more objective, quantitative measure of operative trauma and is increasingly common in postoperative monitoring (Zhang et al. 2013). As this was only a preliminary study, only a small number of animals were included. Moreover, further work is also needed to investigate the systemic inflammatory response to the surgical procedure.

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